Standard Operating Procedure for the Determination of chloride, sulfate, ammonia, nitrate, total phosphorus and total kjeldahl nitrogen in soil

1.0 Scope and Applicability

This method is applicable to chloride, sulfate, ammonia, nitrate + nitrite, total phosphorus and total kjeldahl nitrogen in soil and solid phase materials which may not technically be classified as soils. Such other materials may include: manures; grab samples from settling ponds; solid materials collected from roadways as a result of spills and dumping; Snow Goose fecal; corings from Turkey barn floors; and waste material from cheese producers. The analysis of chloride, sulfate, ammonia, and (nitrate + nitrite) nitrogen are based upon wet extractions which follow a USGS method. Analysis of total phosphorus and total kjeldahl nitrogen are carrier out on dried and ground samples using a modification of EPA method 350.1. The determinative steps of this method use ion chromatography (IC) and flow injection analysis (FIA). The following table list the ranges and detection levels associated with the analytes under study. The ranges may be extended by dilution.

Analyte	range	MDL mg/g	Instrument
ammonia	.05-2ppm	.001	FIA
chloride	5-700ppm	.00157	IC
sulfate	5-700 ppm	.002	IC
nitrate	.05-2 ppm	.0005	FIA
phosphorus	.1-20 ppm	.0457	FIA
TKN	.1-20 ppm	.161	FIA

2.0 Summary of Method

In the Laboratory, nitrogen in soil can be determined in three forms: nitrate, ammonia, and organically bound. Typical soils sampled from an agriculture field would be expected to contain very little nitrogen in the ammonia form. The soil is alive with various types of bacteria and other organisms that use nitrogen in various forms. What ammonia is present would be converted to other forms of nitrogen. Because of the limited ammonia expected most soil labs will dry the soil at room temperature or below 100^{o}C overnight and grind the sample to pass a 0.5 mm screen. A common soil nitrate test would be to make a slurry in a 50 ml beaker and test using a selective ion electrode. A semi-micro Kjeldahl would be run on the soil for TKN analysis.

Nitrate being water soluble will pass through the ground at a rate dependent on the precipitation and permeability of the soil. Nitrogen fixed in an organic form will be attached to the humus within the soil, mostly within the A horizon of the soil profile. To have a useful value for soil nitrogen it is important to know from what depth the sample was obtained. It is customary to use a soil probe (a 2-3 foot long hollow tube about 1 inch in diameter to sample the soil. Ideally the analysis should report the nitrogen

content at a specific depth from which the soil was obtained. This is usually done at 6 inch intervals such as: 0-6 inches; 6-12 inches etc.

Most of the soil samples we receive are not related to a nitrogen determination which will be used in a mathematical model with yield projections for a specific crop such as Spring Wheat. The soil samples we see are a result of some mishap, either an accidental spill or intentional dumping of a substance or something else. Due to the nature of these samples the ammonia levels may be very high. Drying these samples prior to grinding will obviously be a source of error. The following procedure was developed to minimize analyte loss and provide uniformity in reporting results.

A soil sample with several analytic requests should be handled according to one or more of the following extraction procedures.

> Chloride and/or sulfate analysis: A simple water extract can be used.
> Ammonia and/or nitrate analysis: use acidified sodium chloride extract.
> TKN and/or Total Phosphate analysis: The dried and ground soil sample can be digested with sulfuric acid in a block digestor.

The wet extraction for this method is divided into two parts. For the analysis of chloride and sulfate a 100 g sub-sample is placed into a 2 L plastic container and 1000 ml of reagent water is added. The container is sealed and rotated for 1 hour. At the end of the hour the contents are allowed to settle then filtered through course filter paper into a 200 ml plastic bottle. The bottle is stored at 4 deg C until time for the analysis. The chloride and sulfate analysis is carried out on the IC after filtering with a 0.45 micron syringe filter.

Extraction for the analysis of ammonia and (nitrate + nitrite) nitrogen requires a 100 g sub-sample to be placed into a 2 L plastic container with 100 g of sodium chloride, 8 ml of 6N Hydrochloric acid and 950 ml of reagent water. The container is sealed and rotated for 1 hour. At the end of the hour the contents are allowed to settle and the liquid is filtered thru course filter paper into a 200 ml bottle and stored in the refrigerator at 4 deg C until the time of analysis. The ammonia and nitrate samples are diluted 1:10 with reagent water to reduce the salt content from the extraction and run on the FIA using colorimetric procedures.

The ammonia reacts with alkaline phenol, then with sodium hypochlorite to form indophenolblue. Sodium nitroprusside (nitroferricyanide) is added to enhance sensitivity. The absorbance of the reaction product is measured at 630 nm and is directly proportional to the ammonia concentration.
The nitrate analysis uses a cadmium column to reduce the nitrate to nitrite which reacts with sulfanilamide and n-(1-naphthyl)-ethylenediamine dihydrochloride to produce a

colored dye that is measured at 520 nm on the FIA.

The remaining sample is weighed and air dried in order to determine the percent solids. The dried sample is ground in a soil grinder to pass a 10 mesh screen. Approximately 0.1 g of the ground sample is digested in duplicate with sulfuric acid and copper as the catalyst using a block digestor and 75 ml tubes. The digestion will start at 160 deg C for thirty minutes then ramp to 360 deg C for two and one half hours. Following the digestion the tubes are cooled and the volume is reconstituted with reagent water. The samples are vortexed and poured into autosampler tubes for analysis on the FIA. Phosphorus is converted to the ortho form during the digestion. On the FIA this reacts with ammonium molybdate and antimony potassium tartrate in an acid medium to form an antimony-phosphomolybdate complex. This complex is reduced to a blue-colored complex by ascorbic acid. Absorbance is proportional to phosphorus concentration and is measured at 880 nm.
TKN's are measured using the ammonia phenate chemistry described in (Ref 16.1). A slight modification to this chemistry includes increasing the strength of the sodium hydroxide EDTA buffer to compensate for the high acid in the sample digest and increasing the sodium nitroprusside concentration which is used to enhance the color. The TKN results derived from this procedure will include the ammonia nitrogen, not lost thru drying the sample, and the nitrogen bound in organic form. It will not include the nitrogen in nitrate form. All results will be reported or corrected to a dry weight basis.

3.0 Definitions

Calibration blank - A volume of reagent water fortified with the same matrix as the calibration standards, but without the analytes.

Flow Injection Analysis (FIA) - references the Lachat equipment listed in (sec 6.1).

Ion Chromatography (IC) - a determinative procedure using an anion column and a conductivity detector.

Laboratory Fortified Blank (LFB) - An aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

Laboratory Reagent Blank (LRB) - Reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

Material Safety Data Sheet (MSDS) - Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

Method Detection Limit (MDL) - The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero. Defined according to section 16.4.

Quality Control Sample (QCS) - A solution containing a known concentration of the analyte, usually from a source outside the laboratory and independent of your standards. The purpose of this solution is to verify that your standards are correct.

Reagent water - Water which has been run thru a reverse osmoses system and then thru de-ionization cartridges so that the final conductivity of the water is 17 meg ohm.

Stock Standard Solution - A concentrated solution containing the method analyte prepared in the laboratory using a purchased standard reference material, ideally traceable to NIST.

4.0 Interferences

Sample which co-elute with chloride or sulfate on the IC will produce errors in determinations. Samples to be run on the FIA may have to be filtered with finer filter paper if particulate matter is still present. Samples which contain oily film will coat the cadmium column and interfere with the nitrate analysis. Some forms of nitrogen and/or phosphorus may not be completely digested. High concentrations of ammonia in a moist sample may be lost during soil drying with the result that the TKN values may be under reported.

5.0 Safety

WARNING: The handling of phenol in this method is subject to extra care as it can cause server burns on the skin. It can also be absorbed directly through the skin. Exposure of large surface areas of the skin have been linked to death. Brain damage and other neurological problems have also been attributed to inhalation of vapors. Cardiac arrest is also a possibility. Use protective gloves and work under the hood while preparing this reagent. When running this chemistry on the FIA make sure the vent hood over the waste sink is operating and water is running down the sink.

The nitrate chemistry employs the use of a cadmium column. Cadmium has been implicated as a cancer causing agent. Wear protective gloves when re-packing cadmium columns. Normal laboratory precautions should be observed in handling other reagents in this method.

During the grinding of soils considerable dust can be generated depending on soil type. As a precaution a dust mask should be worn.

This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets (MSDS's) should be available to all personnel involved in these analyses.

6.0 Equipment and Supplies

Note: Brand names, suppliers, and part numbers are cited for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using equipment and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.

Instrumentation

6.1 Lachat QuikChem 8000 Flow Injection Analyzer

6.1.1 96 place random access autosampler
6.1.2 Pneumatic pump
6.1.3 Colorimeter with:
10 mm flow cell
520 nm interference filter for nitrate
880 nm interference filter for phosphorus
630 nm interference filter for ammonia and TKNs

6.1.4 Heaters set for 60 deg C for ammonia and TKN, 37 deg C for phosphorus

6.1.5 reaction modules for: ammonia, nitrate + nitrite, phosphorus, TKN

6.1.6 Sample loops. Ammonia 190 cm of .022 inch dia. teflon tubing
TKN 40 cm of .032 inch dia. Teflon tubing
Nitrate + nitrite 190 cm of 0.022 inch dia. Teflon tubing
Phosphate 18 cm of 0.032 inch dia. Teflon tubing

6.2 Gateway 2000 P5-60 computer
6.3 NEC Multisync 3FGe Monitor
6.4 HP 3Si printer
6.5 Omnion Software (ver. 2.0 Jan 99)

6.6 Analytical balance capable of accurately weighing to the nearest 0.0001g
6.7 Analytical balance capable of accurately weighing to the nearest 0.01 g
6.8 Glassware - Class A volumetric flasks and pipets as required
6.9 Dionex DX500 IC unit
  6.9.1 AG14 guard and AS14 anion column (4X250 mm), 30 min run time.
  6.9.2 50 mM borate eluent, 1.5 ml/min.
  6.9.3 Chloride/sulfate water method using a gradient of 4% to 30 % borate.
  6.9.4 Electronic suppression using external water source, 300 ma current.
6.10 DC-2 soil grinder: Custom Laboratory Inc., Orange City, FL.
6.11 Lachat 46 place digestion block: Lachat Instruments Div. Zellweger Analytics, Milwaukee, WI
6.12 Rotator, Model 3740-12-BRE-TM. Associated Design & MFG Co.; Alexandria, VR 22314.

7.0 Reagents and Standards

7.1 Ammonia reagents for FIA

7.1.1 Sodium Phenolate:
CAUTION: wear gloves when handling phenol as you can burn your skin. In a 2 L beaker add 176 ml of liquefied phenol to 1200 ml of reagent water. While stirring, slowly add 64 g of NaOH. Cool and dilute to 2 L. Degas with Helium.

7.1.2 Sodium Hypochlorite:
In a 1 L beaker containing 400 ml of reagent water add 500 ml of regular Clorox bleach (5.25%). Add reagent water to make 1 L of solution. Degas with He.

7.1.3 Sodium Nitroprusside:
In a 2 L beaker add 4 g of sodium nitroprusside ($Na_2Fe(CN)_5NO{\cdot}2H_2O$) to 2 L of reagent water. Mix to dissolve. Degas with Helium.

7.1.4 Buffer:
In a 2 L beaker add 100 g EDTA (Ethylenediaminetetraacetic acid, disodium salt dihydrate):
$NaO_2CCH_2N(CH_2CO_2H)CH_2CH_2N(CH_2CO_2Na)CH_2CO_2H{\cdot}2H_2O$, 19.8 g sodium hydroxide (NaOH) . Mix to dissolve. Degas with Helium.

7.1.5 Carrier:
Use degassed regent water.

7.2 Nitrate Reagents for FIA

7.2.1 Ammonium chloride buffer (alternative recipe):

To 500 mL of deionized water in a 2 L beaker add 210 mL of concentrated HCl, 190 mL of concentrated ammonium hydroxide (NH_4OH) and 2.0 g of EDTA. Dissolve all of the above and bring the solution to a volume of 2 L.

7.2.2 Color Reagent: To approximately 1600 mL of distilled water add 200 mL of conc. H_3PO_4. Add 80 g of sulfanilamide and dissolve completely. Dissolve 2.0 g N-1-Naphthylenediamine dihydrochloride and dilute to two liters. Store in a dark bottle. This reagent is stable for one month. Degas with helium.

7.3 Phosphorus Reagents for FIA

7.3.1 Stock Ammonium Molybdate Solution
In a 1L volumetric flask dissolve 40.0 g ammonium molybdate tetrahydrate [$(NH_4)_6Mo_7O_{24}\bullet 4H_2O$] in approximately 800 ml of water. Dilute to the mark and mix with a magnetic stirrer for at least four hours. Store in plastic and refrigerate.

7.3.2 Stock Antimony Potassium Tartrate Solution
In a 1L volumetric flask, dissolve 3.0 g antimony potassium tartrate (potassium antimony tartrate hemihydrate $K(SbO)C_4H_4O_6\bullet 1/2H_2O$ in approximately 800 ml water. Dilute to mark and mix with a magnetic stirrer until dissolved. Store in a dark bottle and refrigerate.

7.3.3 Molybdate Color Reagent
In a 2L flask, add about 1L of water. Add 144 ml of stock antimony potassium tartrate solution and 426 ml of stock ammonium molybdate solution. Dilute to the mark and stir until mixed. Degas with Helium.

7.3.4 Ascorbic acid
In a 1L beaker dissolve 60.0 g ascorbic acid in about 900 ml of water. Degas with Helium. Add 1.0 g of sodium dodecyl sulfate ($CH_3(CH_2)_{11}OSO_3Na$). Mix with a stir bar and dilute to the 1 liter mark. Prepare fresh weekly more frequently if problems arise.

7.3.5 Carrier
In a 2L beaker dissolve 63.4 g K_2SO_4, and 55 ml conc. H_2SO_4. Stir to dissolve and degass with helium.

7.3.6 Sodium Chloride/Sodium Hydroxide Soln.
In a 2L beaker dissolve 320 g NaCl and 40 g NaOH in about 1200 ml of water. Dilute to the 2 liter mark and degas with Helium.

7.4 TKN Reagents for FIA

7.4.1 Buffer:
In a 2 L beaker add 27.2 g EDTA (Ethylenediaminetetraacetic acid, disodium salt dihydrate): $NaO_2CCH_2N(CH_2CO_2H)CH_2CH_2N(CH_2CO_2Na)CH_2CO_2H \cdot 2H_2O$, 100 g sodium hydroxide (NaOH), and 25.44 g of disodium hydrogen phosphate (Na_2HPO_4) or 48 g of disodium hydrogen phosphate heptahydrate ($Na_2HPO_4 \cdot 7H_2O$). Mix to dissolve. Degas with Helium.

7.4.2 Carrier: use degassed reagent water.
7.4.3 Sodium Phenolate: (same as 7.1.1)
7.4.4 Sodium Hypochlorite (same as 7.1.2)
7.4.5 Sodium Nitroprusside (same as 7.1.3)

7.5 Digestion Reagent for use with block digestor.
In a 1 L beaker dissolve 89 ml of concentrated sulfuric acid (H_2SO_4), 89 g of potassium sulfate (K_2SO_4) and 4.87 g of cupper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) in about 600 ml of reagent water. When completely dissolved bring to a final volume of 1 L with reagent water.

7.6 Standards

7.6.1 Stock Nitrite Standard (100 ppm N/L as NO_2). Dissolve 0.4926 g of sodium nitrite ($NaNO_2$) in approximately 800 mL distilled water and dilute to 1 liter. Prepare the nitrite stock solution fresh daily as it is not stable.

7.6.2 Stock Nitrate Standard (1000 ppm N/L as NO_3)
Dry potassium nitrate (KNO_3) for 24 hours at 105 ^oC.
Store in a desiccator until ready for use. Dissolve 7.2674 g (Fisher primary grade KNO_3 Standard containing 13.76% N or equivalent nitrate standard) in 800 mL distilled water. Add 2 mL chloroform and bring to volume. Store in a dark bottle. The reagent is stable for 6 months.

7.6.3 Ammonia spike solution (1000 ppm N)
In a 1 L volumetric flask dissolve 3.8188 g of NH_4Cl that has been dried for two hours at 105 deg C in about 800 ml of reagent water. Bring to a final volume of 1 liter.

7.6.4 Chloride and Sulfate spike solution (1000 ppm Cl and SO_4)
Dissolve 1.6484 g of NaCl and 1.3757 g of $(NH_4)_2SO_4$ in reagent water.

Bring to volume using a 1 L volumetric flask. Store at 4 deg C.

7.6.5 Combined TKN/PO_4 Stock Standard (1000 mg N/L, 1000 mg P/L)

In a 1 L volumetric flask dissolve 3.819g ammonium chloride (NH_4Cl) that has been dried for two hours at 105°C and 4.3938g KH_2PO_4 in about 800 ml deionized water. Bring to volume. Invert to mix.

7.6.6 Working Standard (100 mg N & P/L)

In 200 ml volumetric flasks add 20 ml of the stock standard (7.6.5). Bring to a volume with reagent water and invert to mix.

Add the following amounts of working standard to the 75 ml digestion tubes and digest with samples.

8.0 Sample Collection, Preservation and Storage

Samples brought to the lab should be in air tight containers to eliminate the loss of volatile ammonia. To minimize any bacterial conversion of ammonia to nitrate the sample should be stored at 4 deg C and analyzed as soon as possible. Because this analysis is not an EPA procedure and/or restricted by a holding time and our primary support is for water analysis, samples will frequently remain shelved until time permits analysis. It is the responsibility of the login staff upon receipt and tagging of a soil sample to place it in the refrigerator and notify the analyst that soil samples have been received.

9.0 Quality Control

9.1 Initial Demonstration of performance for soil digests and analysis of TKN & PO4. The Initial linear calibration range needs to be established over the range of analysis. At least three standards and a blank standard must be used. In the event of a non-linear response additional standards will need to be run in order to quantify the curve. A quality control sample will be run after the initial calibration and must run within +-10% of true value. If the QCS does not run within 10% of true value then the source of the problem must be identified and corrected before analyzing samples. In the event that no problem can be identified the run should be continued and the other QC samples reviewed. Each tube is a separate digest and it is possible for one QCS to fail and the others to pass. The method detection level needs to be established using historic duplicate data carried thru the complete digestion and analysis. MDL is calculated as:

MDL = (t) * (s)

Where t is the t value from the Student's t Table using the 99% confidence level and n-1 degrees of freedom. The standard deviation s, is calculated using "n" duplicates described in the procedure in Standard methods (sec 16.5).

$s = ((|\sum d|)/n)/1.128$ where n = the number of pairs of duplicates
d = the difference between the pair
1.128 is constant related to duplicate data

The MDL should be calculated on the lowest category of means.

9.2 Assessing Laboratory Performance
A mid range calibration standard must be run after every 25th sample. If the CS is within 5% error the analysis may proceed. If the error is between 5 and 8% the calibration must be re-established before continuing. If the CS is over 8% error the calibration must be re-established and all samples following the last acceptable calibration standard must be rerun.

9.3 Assessing analyte recovery and data quality
In the case of chloride, sulfate, ammonia and nitrate analysis one sample in each group of ten or less must be spiked with the analyte during the extraction step (11.1 and 11.2). 10.00 ml of the 1000 mg analyte/L Stock solutions (7.6.2, 7.6.3 or 7.6.4) should be added to the extraction container. This will provide a spike of 10 mg analyte /L. This weight per volume spike amount will be corrected to a weight/weight amount as described in (sec 12.2). The percent recovery for the liquid extracts (chloride, sulfate, ammonia, and nitrate) will be calculated as:

$R = ((C_s - C)/s))*100$

Where R = percent recovery
C_s = fortified sample concentration (mg/g dry wt)
C = sample background concentration (mg/g dry wt)
s = concentration of analyte added to sample (mg/L converted to mg/g dry wt.

Spiked samples which fall outside the control limits when other control factors such as: a good calibration curve, quality control samples, duplicates, and the mid range check standards are running correctly indicate a sample matrix or solution problem. If available Reference Materials should also be run to verify performance.

All soil samples for TKN and PO4 will be run in duplicate. Evaluation of acceptable performance will be based upon the difference of the duplicates.

Duplicates which differ by more than 4 standard deviations are considered unacceptable. Either result may be incorrect. A second digest in duplicate will need to be made to evaluate which of the first two duplicates is correct. If the results of the second run agree with one the first results then, the mean of the three in agreement will be reported. If all four results are random then, the variation will be assumed large for this sample and all four results will be used and the mean reported out.

The standard deviation used to evaluate the means was found to increase with the mean of the TKN and PO4 results (see section 17). Using a second order regression on the standard deviation vs the mean value of TKN or PO4 it is possible to calculate an expected standard deviation associated with a given mean. A window 2 Std. Dev. above and below this mean is used to evaluate the dispersion between the duplicates. This will provide a 95% confidence window.

Individual values will be entered into an Excel spreadsheet. The Excel program will calculate a mean. This mean will be used to calculate a Std. Dev. using the regression equations listed in section 17. The difference between the duplicates will be calculated and compared to four times the standard deviation. The following comparisons will be made. Where D is the difference between the duplicates. See section 12.6 for calculations.

If $D = < 4\sigma$ Then accept result and report the mean

If $D > 4\sigma$ Then reject result, do not report mean, re-digest sample

10.0 Calibration and Standardization for TKN and PO_4 analysis

Prepare seven standards, including a calibration blank, as outlined in section 11.5.7. Calibration is done by the automated colorimetric procedure in section 11.6. A calibration curve is fitted to the calibration solutions concentration/response data using computer based regression curve fitting techniques. The curve should be linear, but tends to run second order. (see section 17), and will run with a correlation coefficient of 0.999 or better. Failure at this point may be corrected by dropping one or two standards if they are serious outliers or rerunning the standards. If this does not correct the problem other solutions should be investigated. A more likely cause would be flow problems caused by obstruction in reagent lines or worn pump tubes. All standards will be digested. A least one quality control sample will also be digested and must run within the established control window.

11.0 Procedures

Extractions

In the sample extraction procedures, if possible the sample should be mixed as is. A random representative sub-sample of 100 g should then be used for the liquid extraction step(s). Weigh up a Duplicate sub-sample for a duplicate analysis and weigh up a separate duplicate sample for a spiked sample if the original sample size is sufficient. If the sample size in not sufficient a reduced volume extraction should be used. Reduce all units by a factor of 10 and use a 200 ml plastic bottle for tumbling or mixing. The remaining soil sample should be air dried and ground with the soil grinder to pass a 10 mesh screen. Discard stones in both sample weighings and after the grinding step.

11.1 Simple Water Extraction for: (Cl^-,SO_4^{-2})

11.1.1 Place a 2 L plastic bottle directly on a two place balance and tare.

11.1.2 Mix the soil and select several sub-samples randomly from the sample jar until you have approximately 100 g of sample. Record the exact weight.

11.1.3 Weigh up a duplicate.

11.1.4 Weigh up a different duplicate to be used for a spiked sample.

11.1.5 Add 1000 ml of reagent water to each 2 L bottle.

11.1.6 Add 10.0 ml of 1000 ppm chloride and sulfate spike solution (7.6.4) to the spiked sample.

11.1.6 Place tightly capped bottles on the rotating mixer (6.12) for one hour.

11.1.6 Remove bottle from the rotator and allow the mixed samples to settle.

11.1.7 Carefully filter the liquid into a clean 200 ml bottle. Refrigerate for later use. Treat this sample as you would for a chloride or sulfate analysis. Follow the procedures outlined in SOP I-1-5 (Standard Operating Procedure for the analysis of Fluoride, Chloride and Sulfate in water).

11.2 Acidified Sodium Chloride Extraction (NH_4^+,NO_3^{--})

11.2.1 Place a 2 L plastic bottle directly on a two place balance and tare.

11.2.2 Add 100 g (+- 1 g) of reagent grade NaCl and tare the bottle.

11.2.3 Mix the soil and select several sub-samples randomly from the sample jar until you have approximately 100 g of sample. Record the exact weight.

11.2.4 Add 950 ml of reagent water to each 2 L bottle. This volume compensates for the sodium chloride and HCl added.

11.2.5 Add 8.0 ml of 1:1 hydrochloric acid.

11.2.6 Weigh up a duplicate sample following (11.2.1 thru 11.2.5).

11.2.7 Add 10.0 ml of 1000 ppm nitrate (7.6.2) and ammonia (7.6.3) solutions to the spiked samples.

11.2.8 Place tightly capped bottles on the rotating mixer (6.12) for one hour.

11.2.9 Remove bottles from the rotator and allow the mixed sample to settle.

11.2.10 Carefully filter the liquid into a clean 200 ml bottle. Refrigerate for later use.

11.2.11 Dilute these sample 1:10 and treat these sample as you would for nitrate and/or ammonia water analysis using SOP I-1-2 (Standard Operating Procedure for the Determination of Ammonia) and SOP I-1-10 (Standard Operating Procedure for the Analysis of Nitrogen in Nitrate or Nitrite form).

11.2.12 The Percent Solid Content of the soil (ie 87% dry wt) will need to be entered into the LIMS computer before the wet extract results can be entered. The mg /g wet soil will be converted to mg/g dry soil.

11.3 Procedure for determining percent solid content of soil.

11.3.1 After procedures 11.1 and 11.2 have been completed, if required, weigh the sample container and contents to 0.00 g and record the weight.

11.3.2 scrape out the soil sample from the container onto a clean sheet of paper or a glass pie plate. Place the sample on the lab bench away from chemical interferences.

11.3.3 Let the soil air dry overnight or longer if needed. If the soil sample has standing water, place it in a beaker and dry it in a low temperature oven

(around 30 deg C). Record the date and time of the start of the drying time and note the conditions such as air dry or oven dry and temperature.

11.3.4 When the soil sample has completely dried, carefully return the dried soil to the original container.

11.3.5 Re-weigh the dry soil and container.

11.3.6 Determine the weight of the container.

11.3.7 Using the calculation program which is part of the LIMS data entry menu for the analyte {6620} (% solids) enter the required information on wet weights, dry weights, container weight, drying times and temperature.

11.3.8 Samples may be stored at room temperature until needed for further analysis.

11.4 Procedure for grinding samples

11.4.1 Set the soil grinder up in the grinding room within the garage.

11.4.2 Use a dust mask when grinding. Use the air hose and blow out the mill and receiving tray.

11.4.3 Replace the receiving tray in the bottom of the mill. Turn on the mill.

11.4.4 Add the complete sample at one time if possible and close the top lid. For larger samples grind one or more portions separately.

11.4.5 When soil has been ground to a uniform grind open the gate and let the soil fall into the receiving tray.

11.4.6 Turn the mill off. With your hand over the lower opening of the receiving tray gently shake the tray back and forth so that all fine material falls thru the 10 mesh screen leaving only larger pebbles and rocks in to top part.

11.4.7 Discard the rocks into the waste basket.

11.4.8 Removing your hand from the lower part of the tray gently pour the ground contents into the original container or a new one. If using a new container make sure the login numbers are correctly transferred to the new containers.

11.4.9 If more than one grinding is required for large samples, combine all the

ground portions into the same container. Rotate the container to mix the contents completely.

11.5 Procedure for digestion of the ground soil sample.

11.5.1 Weigh up approximately 0.1 grams of soil into the 75 ml TKN digestion tubes using the four place balance and weigh boat in the Feed and Fertilizer lab. Record the exact weight to four places. Use enough sample to have four significant figures in your weight. For example 0.1045 g instead of 0.0996 g.

11.5.2 Duplicate each sample with a separate weight.

11.5.3 Start with and after every 10 weights, weigh up a soil check sample.

11.5.4 Leave the last 8 of the 46 tubes empty for standards.

11.5.5 Add 7-10 boiling chips to each tube (Chemware PTFE boiling stones, Fisher Scientific Co. A1069103).

11.5.6 Add 7.5 ml of the digestion solution containing the Cu catalyst (7.5).

11.5.7 Add the following amounts of working standard (7.6.6) to the 75 ml digestion tubes.

ml of 100 mg/L Working Standard	Digested Standards ppm
8	40
4	20
2	10
1	5
.4	2
.2	1
.1	0.5
.02	0.1
0	0

11.5.8 Vortex all tubes.

11.5.9 Place all tubes into the Lachat block after it has warmed up to 160 deg C.

11.5.10 Press the start button on the control panel of the block digestor

11.5.11 Add the cold fingers when the alarm sounds (30 min) and press the button to continue the temperature ramp.

11.5.12 WARNING (these tubes will be hot: 360 deg C). When the digestion is complete (2 ½ hr) remove the tube racks from the block and place in the rack holder until slightly cooled: the funning sulfuric acid fumes should have abated.

11.5.13 Add 19 ml of reagent water and vortex the tubes to get the dissolved salts back into solution.

11.5.14 Pour up the standards and samples into auto sampler vials, parafilm and place into the refrigerator until ready for analysis on the FIA.

11.5.15 Settling of fine grains will occur overnight. If samples are still turbid, filter with a 1 micro syringe filter (Gelman glass Acrodisc P/N 4523).

11.6 Procedure for running TKN and PO4 on the FIA.

11.6.1 Turn on the computer and all other components. Set the TKN heater to 60 deg C and the PO4 heater to 37 deg C.

11.6.2 Install the TKN and PO4 manifold boards, interference filters and sample loops. Connect all pump tubes. Pump reagent water and check for leaks

11.6.3 Place standards into the autosampler in descending order. Load all samples.

11.6.4 Open the proper method (soilTKTP.met) and Tray Table (TKN.tra) on the FIA computer.

11.6.5 Place all feed lines into the reagents and pump until a stable base line is obtained.

11.6.6 Transfer the work list to a 3 ½ disk, by exporting the work list from a LIMS connected computer.

11.6.7 Import the work list from the 3 ½ inch disk to the FIA computer using the Start menu and selecting: “receive log numbers”. Select “2. Final Export to Instrument”. At the prompt provide a file name such as the current date: (YYMMDD##) 0101090A. At the second prompt indicate that your

first sample will start in position 9 following the standardization.

11.6.8 Exchange the current work list in the FIA computer memory with the new one imported in 11.6.7 by selecting File, “import Tray” and then selecting the file name used in 11.6.7 and selecting OK.

11.6.9 Check the tray table (work list) on the FIA computer for completeness of the transfer, dilutions that were made and deleting any unwanted items left over from the previous tray. All samples will have a dilution factor of 0.02. Enter the weights used for the digestion. Replace the old tray by saving the newly transferred one: “File”, “Save Tray”.

11.6.10 Start the analysis by clicking the “Run Tray” button on the main tool bar. On the next window click catalog on the file name line. On the next window type in your file name you used in 11.6.7. Click OK.

11.6.11 Calibration should start automatically. After the calibration has completed verify that the calibration curve is acceptable.

11.6.12 Check to see that the QCS is running within 10% of true value.

11.6.13 When the analysis is complete remove all pump lines from the reagents and place in reagent water for 5 or 10 min. Remove lines from the water and pump dry. Release pump tubes from the pump. If needed lines may be cleaned by pumping Contrad 70 Detergent (Fisher Scientific 04-355) through them for 5 min followed by reagent water for 5 minutes.

11.6.14 Turn off the pump and all modules. Release the pump tube cassettes.

11.6.15 Print the calibration curve. From the tool bar click the graphic of the Calibration curve ”Review”. Select: “Print”, “Current Analyte”.

11.6.16 Print the custom report. From the tool bar click “Custom”, “Print”, “OK”

11.6.17 Enter the duplicate data into the LIMS computer.

11.6.18 Using the evaluation procedure explained in (sec 12.6) decide which samples if any will need to be re-digested and rerun.

11.6.19 Instrument setup parameters:

	TKN	PO4
Chemistry	direct	direct

Injection to peak start	20	16
Peak base width	75	50
% width tolerance	15	15
Threshold	8800	2000
Calibration fit	2nd order	2nd order
Force thru zero	No	No
Cycle period	80	80
Sample reaches first valve	22	22
Load period	12	12

11.7 Procedure for running ammonia and nitrate on the FIA.

Follow the procedure listed in SOP I-1-2 (Standard Operating Procedure for the Determination of Ammonia) and SOP I-1-10 (Standard Operating Procedure for the Determination of Nitrogen in Nitrate or Nitrite form).

11.8 Procedure for running chloride and sulfate on the IC.

Follow the procedure listed in SOP I-1-5 (Standard Operating Procedure for the analysis of Fluoride, Chloride and Sulfate in Water).

12.0 Data Analysis and Calculations

12.1 Dry Content of soil

Dry content = (wt of dry soil)/(wt. of wet soil)

12.2 Liquid Extraction results

Results for chloride, sulfate, ammonia, and nitrate will be in terms of mg analyte/L. This will need to be converted to mg analyte / g soil. Assume a chloride result of 130.5 mg Cl/L was obtained from a liquid extract which used 101.34 g of wet soil.

$$\frac{130.5 \text{ mg Cl}}{1\text{L}} \times \frac{1\text{L}}{101.34 \text{ g wet soil}} = 1.288 \text{ mg Cl/g wet soil}$$

This calculation may be done by a calculator or can be handled by the Lachat and Dionex software, if the soil extraction weights are entered. This result will be further adjusted to dry wt by the LIMS computer. Assume this sample had a dry content of 87.6%.

$$\frac{1.2877 \text{ mg Cl}}{\text{g wet soil}} \times \frac{1 \text{ g wet soil}}{0.876 \text{ g dry soil}} = 1.460 \text{ mg Cl/ g dry soil}$$

If a reduced volume extraction was used, say 10 g of wet soil in 100 ml of water then a correction factor of 10 will need to be made for the calculations. The concentration in mg analyte/L will be the same but, the weight will need to be multiplied by 10 to indicate what weight would have been used for a L of extraction solution. For example: if 10.13 grams of wet soil was used with 100 ml of extraction solution and the chloride value read 130.5 Cl/ L.

$$\frac{130.5 \text{ mg Cl}}{\text{L}} \times \frac{100 \text{ ml}}{10.13 \text{ g wet soil}} \times \frac{1 \text{ L}}{1000 \text{ ml}} = 1.288 \text{ mg Cl/ g wet soil}$$

12.3 Calculation of the spike amount for liquid extracts

Although the mg analyte/L will be the same for each sample spiked, the spike amount will be different as it depends on the weight of the spiked sample. For example: 10 ml of 1000 mg Chloride/L was added to a extraction containing 107.87 g of soil in 1L.

Spike amount = (10 mg Cl/L) / (107.87 g wet soil /L) = 0.0927 mg Cl/g wet soil

This "spike amount" will need to be calculated manually or with the aid of a spread sheet. The LIMS will not do this. The spike amount will now be corrected for moisture.

Spike amount = (0.0927 mg Cl/ g wet soil) X (1 g wet soil/ 0.876 g dry soil)

= 0.1058 mg Cl/g dry soil

12.4 Calculations for TKN and Phosphorus results from acid digests

The soil weight and volume used for the acid digest can be entered into the Lachat work list which will correct the results from mg analyte/L to mg analyte/ g dry soil. For example 0.1243 g of dry soil was used for a digest which was brought to a final volume of 20 ml. This sample produced a result of 18.65 mg P/L.

$$\left(\frac{18.65 \text{ mg P}}{1 \text{ L}}\right) \times \frac{0.02 \text{ L}}{0.1243 \text{ g dry soil}} = 3.00 \text{ mg P / g dry soil}$$

Alternatively the correction to mg analyte per g dry soil could be made using a spread sheet with the actual FIA results expressed in mg of analyte/L. TKN and P results will be entered into the LIMS as mg P/g dry soil or mg TKN/g dry soil.

12.5 Conversions factor which may be useful.

Percent to parts per million: (%) (10,000) = ppm
(1.2 % N) (10,000) = 12000 ppm N
= 12000 mg N/L
= 12 mg N/g

A soil check sample is reported to have 0.2618 % N.

(0.2618 % N)(10,000) = 2618 ppm N = 2.618 mg N/g soil

A reagent you use has 0.005 % nitrogen contamination.

(0.005% N)(10,000) = 50 ppm N = 0.05 mg N/g reagent

12.6 Calculations showing how duplicate precision is used to accept or reject data.

A sample was run in duplicate for TKN and Phosphorus. The results were: (1.287 and 1.416 mg N/g) and (0.206 and 0.291 mg P/g).

	Mean	σ	4σ	Duplicate difference	decision
TKN	1.3515	0.08199	0.328	0.129	accept mean
P	0.2485	0.01948	0.0779	0.085	reject mean

$TKN(\sigma) = 0.0526(1.3515)^2 - 0.0597(1.3515) + 0.0666 = 0.08199$
$P(\sigma) = 0.1693(0.2485)^2 - 0.0035(0.2485) + 0.0099 = 0.01948$

Means which fall outside the range for which control data is available will use the following: TKN means < 0.2 mg/g will use a mean of 0.2 mg/g
TKN means > 3 mg/g will use a mean of 3 mg/g
P means < 0.24 mg/g will use a mean of .24 mg/g
P mens > 0.66 mg/g will use a mean of 0.66 mg/g

13.0 Method Performance

The MDL's for Total phosphorus and Total Kjeldahl Nitrogen from acid digests are based upon actual performance. Ten pair of duplicates for P, with a mean range of (0.240 - 0.268 mg P/ g dry soil) were used. The precision as measured by the std. dev using (sec. 16.5) was 0.0162. The MDL was 0.0457 mg P/g soil. Nine pair of duplicates for TKN

were used spanning a range of (0.279 - 0.361 mg TKN/g dry soil). The precision as measured by the standard deviation was 0.05565. The MDL was 0.161 mg TKN/g dry soil.

Method performance for chloride and sulfate are based upon he MDL's for the IC method and converted from mg analyte/L to mg analyte/ g wet soil. The soil weight was assumed to be 100.0 g. Chloride MDL = (0.157 mg Cl/L)(1L/100 g wet soil) = 0.00157 mg Cl/g wet soil. Sulfate MDL = (0.2 mg SO4/L)(1L/100 g wet soil) = 0.002 mg SO4/g wet soil.

Method performance for ammonia and nitrate are likewise converted from the FIA MDL's. Ammonia MDL = (0.01 mg N/L)(1L/100 g wet soil) = 0.0001 mg N/g wet soil. Nitrate MDL = (0.005 mg N/L)(1L/100 g wet soil) = 0.00005 mg N/g wet soil. Correcting for a 1:10 dilution the MDL is ammonia (0.001 mg N/g); nitrate (0.0005 mg/g).

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14.0 Pollution Prevention

Phenol has a serious potential for pollution and only minimum quantities of sodium phenate should be prepared, as needed. Do no make more than can be used in 3 to 4 weeks. All spent cadmium must be collected and stored until period disposal has been arranged by lab management.

15.0 Waste Management

It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

For further information on waste management consult The Waste Management Manual for Laboratory Personnel and Less is Better: Laboratory Chemical Management for Waste Reduction, both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW., Washington DC, 20036.

16.0 References

16.1 EPA (Aug 93) Method 350.1 (Colorimetric, Automated Phenate)
16.2 Lachat QuikChem Method No. 10-107-06-1-B (Dec 1993) "Ammonia (Phenolate) in Potable and Surface Waters".
16.3 USGS TWRI Book 5, Chapter A1, 1989

16.4 Methods of Soil analysis, Part 2, Chemical and Microbiological Properties, 2nd ed. 1982, chapter 31: Total Nitrogen.
16.5 Standard Methods 19th ed., 1995: 1030C, Precision.

17.0 Tables, Diagrams, flowcharts, and Validation Data

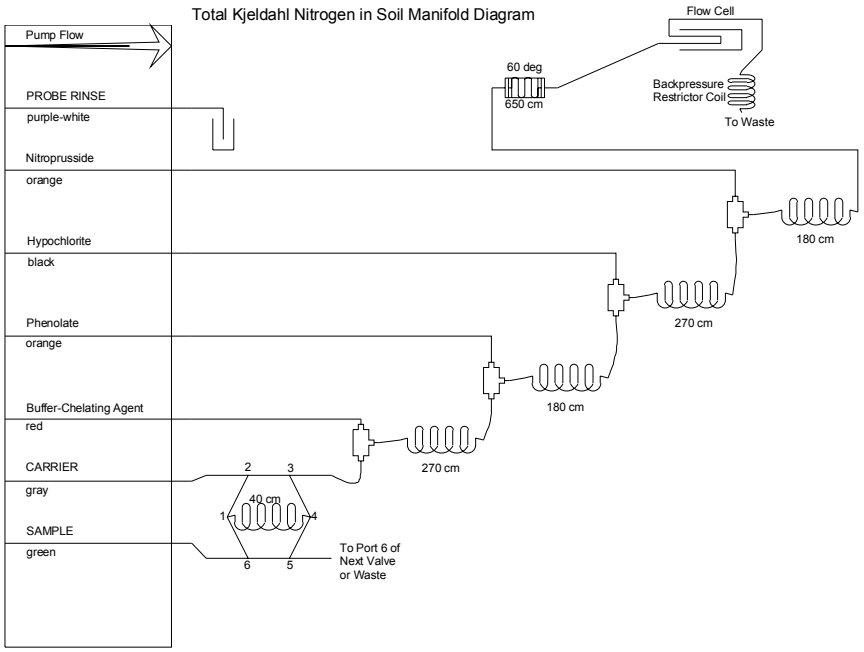
Formulas used for the Excel spread sheet.

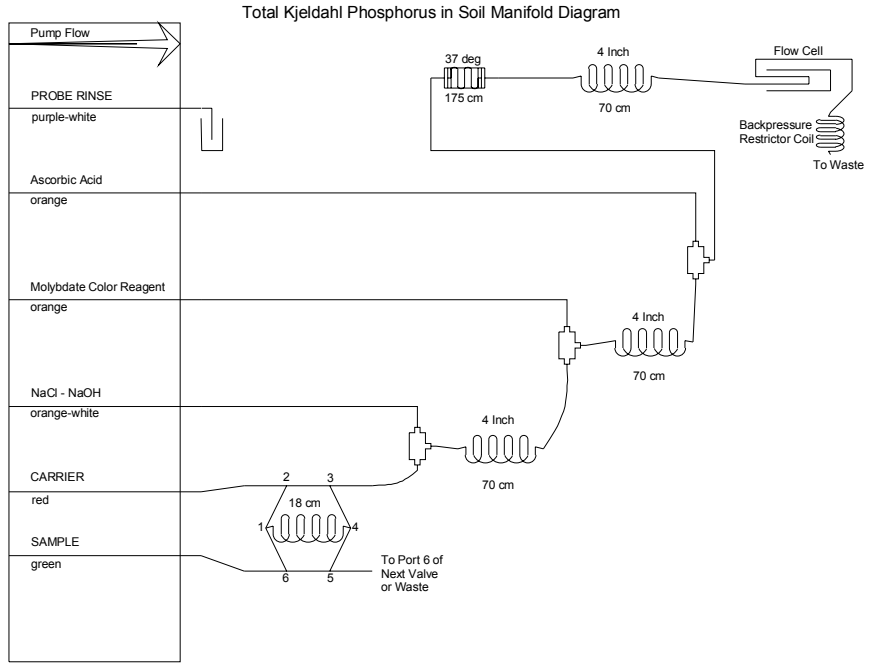
TKN evaluation:

Column	description	formula
A	Log Num	none
B	1st TKN	none
C	2nd TKN	none
D	Mean	=(B2+C2)/2
E	Use Mean	=IF(D2<0.2,0.2,IF(D2>3,3,D2))
F	Std. Dev.	=(0.0526)*(E2)*(E2) - (0.0597)*(E2) + 0.0666
G	4 Std. Dev.	=4*(F2)
H	Dup Range	=ABS(B2-C2)
I	Decision	=IF(G2>H2,"enter data","rerun")

Phosphorus evaluation (lines which change):

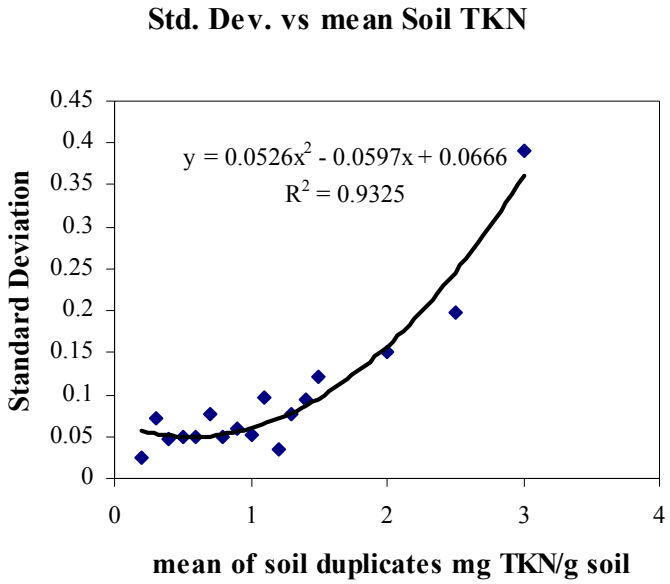
B	1st PO4	none
C	2nd PO4	none
E	Use Mean	=IF(D2<0.24,0.24,IF(D2>0.66,0.66,D2))
F	Std. Dev.	=(0.1693)*(E2)*(E2) - (0.0035)*(E2) + 0.0099


Total Kjeldahl Nitrogen in Soil Manifold Diagram
Pump Flow
PROBE RINSE
purple-white
Nitroprusside
orange
Hypochlorite
black
Phenolate
orange
Buffer-Chelating Agent
red
CARRIER
gray
SAMPLE
green
60 deg
650 cm
Flow Cell
Backpressure
Restrictor Coil
To Waste
180 cm
270 cm
180 cm
270 cm
2
3
40 cm
1
4
6
5
To Port 6 of
Next Valve
or Waste

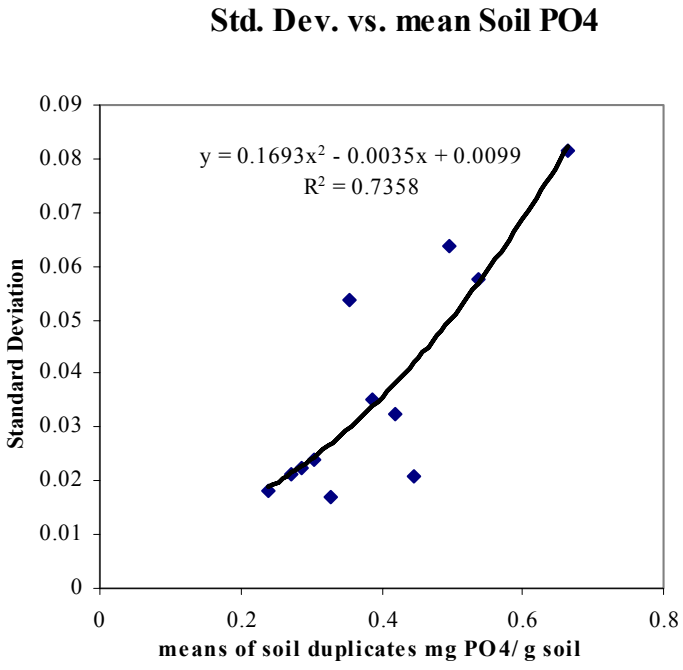

Total Kjeldahl Phosphorus in Soil Manifold Diagram
Pump Flow
PROBE RINSE
purple-white
Ascorbic Acid
orange
Molybdate Color Reagent
orange
NaCl - NaOH
orange-white
CARRIER
red
SAMPLE
green
37 deg
175 cm
4 Inch
70 cm
Flow Cell
Backpressure
Restrictor Coil
To Waste
4 Inch
70 cm
4 Inch
70 cm
2
3
18 cm
1
4
6
5
To Port 6 of
Next Valve
or Waste

Determining Precision from Soil Duplicate Data

lower limit mg N/g soil	Std. Dev.	number in means
0.2	0.0248	3
0.3	0.0711	6
0.4	0.0481	12
0.5	0.0486	10
0.6	0.0497	13
0.7	0.0758	12
0.8	0.0486	10
0.9	0.0595	10
1	0.0526	7
1.1	0.0965	7
1.2	0.0337	6
1.3	0.0757	5
1.4	0.0933	7
1.5	0.1204	6
2	0.1496	8
2.5	0.1986	5
3	0.3902	5



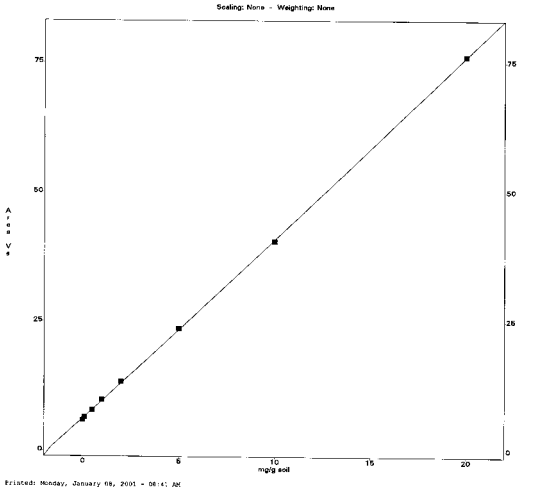
lower limit mg P/g soil	Std. Dev	number in means
0.24	0.018	10
0.271	0.0212	10
0.287	0.0225	10
0.304	0.0239	10
0.327	0.017	10
0.355	0.0536	10
0.386	0.035	10
0.419	0.0326	10
0.447	0.0207	10
0.497	0.0639	10
0.536	0.0574	10
0.665	0.0816	10
2.01	1.099	8



Calibration Curve for Total Kjeldahl Phosphorus from Soil

Lvl	Area	mg/g soil	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Replic SD	Replic % RSD	Residual 2nd poly
1	76202288	20.0	76202288					0.0	0.0	-0.1
2	40496884	10.0	40496884					0.0	0.0	0.9
3	23661022	5.0	23661022					0.0	0.0	-1.3
4	13372696	2.0	13372696					0.0	0.0	-1.9
5	9893795	1.0	9893795					0.0	0.0	+6.5
6	7909183	0.5	7909183					0.0	0.0	2.9
7	6474888	0.1	6474888					0.0	0.0	33.7
8	5951394	0.0	5951394					0.0	0.0	

2nd Order Poly
Conc = -9.817e-017 Area² + 2.936e-007 Area - 1.631e-000
r = 1.0000



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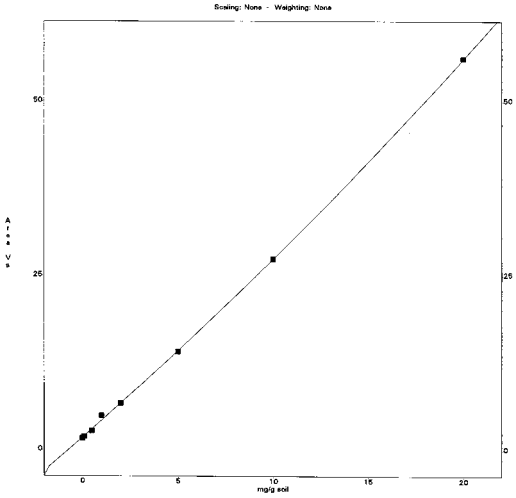
Calibration Curve for Total Kjeldahl Nitrogen from Soil

Lvl	Area	mg/g soil	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Replic SD	Replic % RSD	Residual 2nd poly
1	56112136	20.0	56112136					0.0	0.0	-0.0
2	27412824	10.0	27412824					0.0	0.0	0.0
3	14130919	5.0	14130919					0.0	0.0	1.3
4	6736526	2.0	6736526					0.0	0.0	0.2
5	4961407	1.0	4961407					0.0	0.0	-27.9
6	2768410	0.5	2768410					0.0	0.0	22.9
7	1942869	0.1	1942869					0.0	0.0	53.3
8	1731125	0.0	1731125					0.0	0.0	

2nd Order Poly
Conc = -7.727e-016 Area² + 4.133e-007 Area - 7.525e-001
r = 0.9999



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